Drug Response Evaluation for NF1 Tumors using High Throughput Screening Analysis

Paul Zamora[1], Gabriel Altay[1], Ulisses Santamaria[1,2], Nathan Dwarshuis[1,2], Hari Donthi[1], Chang In Moon[1], Dawn Adams[1], Dana Bakalar[1], Matthew Zamora[1,2]
[1]MoCo Makers, [2]DMV Petri Dish, Maryland
*Corresponding Author – matt@mocomakers.com

The DREA Web Tool to explore qHTS data. Contact matt@mocomakers.com for the login password.

Introduction:

Neurofibromatosis type 1 (NF1) is a genetic disorder in which mutations of the *NF1* gene predispose patients to developing tumors. Many tumors themselves carry mutations in *NF1,* and mutations in *NF1* are associated with resistance to therapeutics and poor treatment outcomes. There are currently no approved treatments for adult patients.

We analyzed a quantitative high throughput screening (qHTS) data set*, consisting of dose/response curves for 1,921 compounds tested against non-tumor reference and plexiform neurofibroma-derived immortalized tumor cells.

We algorithmically combined data per cell line/compound as a score ***S*** that combines compound effectiveness and potency. By comparing a non-tumor reference to each tumor cell line, we established the single-value ΔS_{mean} which is useful for a cross-compound/cross cell line evaluation and identified a panel of promising compounds. The ***ΔS*** analysis may be useful in supporting follow-on pre-clinical development.

* data: https://www.synapse.org/#!Synapse:syn5522627

Methods:

In qHTS, dose-response curves may reflect complete or partial effectiveness. For complete responses, AC50 can be used to compare potency of two or more drugs. This form of analysis breaks down with partial responses and partial effectiveness.

To solve this problem, we present ***ΔS,*** a novel scoring system inspired by receptor pharmacology and here applied to qHTS of NF1 patient plexiform neurofibroma-derived cell lines.

Algorithm

A single score value, **S,** for each drug/cell line combination is defined as the ratio:

$$S = \log \frac{EFF}{\mathrm{AC50}}$$

where drug effectiveness (EFF) is defined as the difference between the response at zero concentration ($\mathbf{Response_{ZERO}}$) and at theoretical infinite concentration ($\mathbf{Response_{INF}}$):

$$\mathbf{EFF = Response_{ZERO} - Response_{INF}}$$

The potency of a compound is defined as the drug concentration at half-maximum response – **AC50.**

The relative effectiveness of a compound is defined as: $\mathbf{\Delta EFF = EFF_{ref}/EFF_{test}}$
The relative potency **ΔAC50** of a compound exposed to two cell lines is:

$$\Delta\mathrm{AC50} = \left[\log \mathrm{AC50}_{ref} - \log \mathrm{AC50}_{test}\right] = \log \frac{\mathrm{AC50}_{ref}}{\mathrm{AC50}_{test}}$$

Combining relative effectiveness and relative AC50 potency into a single number, ***ΔS*** is the difference between the scores $\mathbf{S_{ref}}$ and $\mathbf{S_{test}}$:

$$\Delta S = S_{ref} - S_{test} = \log\left(\frac{EFF}{\mathrm{AC50}}\right)_{ref} - \log\left(\frac{EFF}{\mathrm{AC50}}\right)_{test}$$

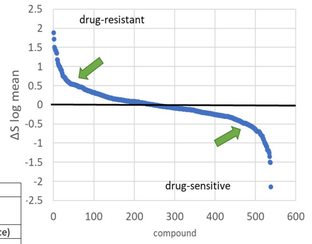
$$\Delta S = \log\left[\left(\frac{EFF_{ref}}{EFF_{test}}\right)\left(\frac{\mathrm{AC50}_{test}}{\mathrm{AC50}_{ref}}\right)\right] = \log\left(\frac{EFF_{ref}}{EFF_{test}}\right) + \log\left(\frac{\mathrm{AC50}_{test}}{\mathrm{AC50}_{ref}}\right)$$

ΔS normalizes drug potency and effectiveness into a single value allowing comparison of drugs with complete and partial responses across drug classes and cell lines.

Drug Selection

Drugs for which 3 or more cell lines were "sensitive" (ΔS_{mean} is < 0.5 relative to the reference cell line) are presented as therapeutic candidates. Compounds where 3 or more cell lines are "resistant" (ΔS_{mean} > 0.5) are also presented. ΔS_{mean} is a mean of all ***ΔS*** values from each compound/cell line combination, omitting cell lines that did not have dose-response curve R^2 of at least 0.8

A ranking of all measured compounds by ΔS_{mean}. Positive values represent drug-resistance, and negative represent drug-sensitivity. Values near zero represent identical response in tumor cell lines and the reference. Green arrows mark inflection point near 0.5 and -0.5



HUMAN CELL LINES USED IN THE ANALYSIS

cell line	immortalized*	source description	neurofibromin status	tumor status
ipnNF95.11c	yes	Peripheral nerve	-/-, +/-	non-tumor (reference)
ipNF06.2A	yes	Plexiform Neurofibroma	-/-	tumor
ipNF95.6	yes	Plexiform Neurofibroma	-/-	tumor
ipNF95.11bC_T	yes	Plexiform Neurofibroma	-/-	tumor
ipNF05.5 (mixed clone)	yes	Plexiform Neurofibroma	-/-	tumor

*viral delivery of human TERT and murine CDK4

The set of cell lines used in the scoring algorithm, including ipnNF95.11c as the reference non-tumor cell line. The other four tumor lines are compared against the reference.

Results:

Compounds to which NF1 cell lines are sensitive (left table) and resistant (right table)

ΔS for each cell line (tumor cell lines)

DNA/cell cycle

name	target	ipNF05.5	ipNF06.2A	ipNF95.11b	ipNF95.6	ΔS mean	ΔS variance	num_lines
Milciclib	CDK1					-0.69	0.42	4
Seliciclib	CDK1				NA	-0.59	0.01	3
BMS-265246	CDK1		NA			-0.57	0.14	3
PHA-690509	CDK2					-0.73	0.54	4
GSK-923295	CENPE	NA				-0.55	0.36	3
SCH-900776	CHEK1					-1.01	0.16	4
CAL-101	PIK3CD					-0.78	0.70	4

Cell membrane receptors/channels

name	target	ipNF05.5	ipNF06.2A	ipNF95.11b	ipNF95.6	ΔS mean	ΔS variance	num_lines
Shikonin	CCR5		NA			-0.97	0.03	3
Gefitinib PR7	EGFR	NA				-1.50	1.54	3
CUDC-101	EGFR					-1.20	1.80	4
Varlitinib tosylate	EGFR					-0.92	0.35	4
Erlotinib HCl	EGFR		NA			-0.61	0.26	3
Pelitinib	EGFR					-0.58	0.81	4
Tivozanib	FLT1		NA			-1.22	0.38	3
Motesanib	FLT1					-0.89	0.78	4
Vargatef	FLT1					-0.70	0.65	4
Piboserod HCl	HTR4					-0.87	0.51	4
BMS-536924	IGFR1	NA				-0.55	0.40	3
GSK-1838705A	IGFR1	NA				-0.52	0.69	3
OSI-632	KDR					-0.53	0.38	4
Clomipramine HCl	SLC6A4					-1.35	0.50	4
Duloxetine HCl	SLC6A4		NA			-1.17	0.21	3
AZ 10606120	P2RX7		NA			-1.10	0.31	3
A 804598	P2RX7		NA			-0.82	0.67	3

PIK3-MTOR pathway

name	target	ipNF05.5	ipNF06.2A	ipNF95.11b	ipNF95.6	ΔS mean	ΔS variance	num_lines
Tricirbine phosphate	AKT1					-1.51	0.81	4
A-443654	AKT1					-0.82	0.04	4
AT-7867	AKT1		NA			-0.51	0.16	3
WYE-354	MTOR					-0.53	1.15	4
3-Methyladenine	PIK3CA	NA				-1.01	0.16	3
GNE-490	PIK3CA					-0.66	0.25	4

non-receptor protein kinase

name	target	ipNF05.5	ipNF06.2A	ipNF95.11b	ipNF95.6	ΔS mean	ΔS variance	num_lines
DCC-2036	ABL1					-0.64	0.12	4
CT-99021	GSK3B		NA			-1.07	0.34	3
IKK-2 Inhibitor VIII	IKBKB					-0.71	0.12	4
BMS-345541	IKBKB					-0.71	0.38	4
MLN-120B	IKBKB		NA			-0.60	0.41	3
Honokiol	NFKB1					-0.54	0.34	4
G7-6983	PRKCA		NA			-0.81	0.95	3
AIM-100	TNK2					-0.54	0.16	4
NCGC00244250-01	TYK2				NA	-0.68	1.42	3

other targets

name	target	ipNF05.5	ipNF06.2A	ipNF95.11b	ipNF95.6	ΔS mean	ΔS variance	num_lines
Temocapril HCl	ACE					-0.53	0.11	4
Staurosporin 1	AHR			NA		-0.70	0.87	3
Sepantronium bromide	BIRC5		NA			-1.07	0.62	3
Tipifarnib	COX10		NA			-0.69	0.49	3
Terriflunomide	DHODH		NA			-0.67	0.21	3
STF-083010	ERN1			NA		-1.27	1.10	3
Ethinylestradiol	ESR1		NA			-0.56	0.60	3
Fluvastatin	HMGCR					-0.80	0.04	4
Pitavastatin calcium	HMGCR			NA		-0.52	0.01	3
Simvastatin	HMGCR					-0.50	0.33	4
Geldanamycin	HSP90AB1					-1.20	0.79	4
Alvespimycin HCl	HSP90AB1					-1.17	0.65	4
Retaspimycin	HSP90AB1					-0.57	0.18	4
Mycophenolic acid	IMPDH1					-0.77	0.04	4
Mycophenolate mofetil	IMPDH1		NA			-0.62	0.06	3
Chlorhexidine	MMP1		NA			-0.74	0.11	3
Delanzomib	PSMD1		NA			-0.71	1.36	3

no clear target

name	target	ipNF05.5	ipNF06.2A	ipNF95.11b	ipNF95.6	ΔS mean	ΔS variance	num_lines
Secoisolariciresinol			NA			-2.15	0.09	3
Verteporfin			NA			-1.03	0.12	3
Amiodarone			NA			-0.63	0.05	3
Emetine			NA			-0.63	0.42	4
Avobenzone			NA			-0.56	0.20	3

Top compounds displaying drug-sensitivity in NF1 tumor cell lines. Red data bars indicate the ΔS for each cell line respective (left to right) in the row.

ΔS for each cell line (tumor cell lines)

DNA/cell cycle

name	target	ipNF05.5	ipNF06.2A	ipNF95.11b	ipNF95.6	ΔS mean	ΔS variance	num_lines
TAK-901	AURKA					0.75	0.18	4
RGB-286147	CDK1		NA			0.85	0.77	3
Dinaciclib	CDK1		NA			0.71	1.46	3
Flavopiridol???	CDK1		NA			0.61	0.06	3
PHA-793887	CDK1		NA			0.53	0.03	3
NU-6027	CDK1					0.51	0.14	4
GN-01910	PLK1			NA		0.53	0.12	3
Rifapentine	PRKDC					0.51	0.09	4
Topotecan HCl	TOP1					0.62	0.05	4
SN-38	TOP1					0.5	0.34	4
Epirubicin HCl	TOP2A					0.9	0.09	4
Idarubicin HCl	TOP2A		NA			0.9	0.03	3
Doxorubicin	TOP2A					0.6	0.05	4
Mitoxantrone	TOP2A					0.6	0.08	4
Indibulin	TUBB					1.44	0.08	4
Noscapine	TUBB					1.35	0.03	4
Vinflunine	TUBB		NA			1	0.62	3
Vinorelbine	TUBB					0.81	0.69	4
Paclitaxel	TUBB					0.67	0.2	4

Cell membrane receptors/channels

name	target	ipNF05.5	ipNF06.2A	ipNF95.11b	ipNF95.6	ΔS mean	ΔS variance	num_lines
AEE-788	EGFR			NA		0.61	0.01	3
Sapitinib	ERBB2				NA	1.88	1.94	3
AST-1306	ERBB2					1.4	0.3	4
ARRY-380	ERBB2					0.58	0.01	4
LY-2874455	FGFR1					0.75	0.08	4
HMSL10084	FLT3		NA			0.52	1.03	3
Tivantinib	MET		NA			0.57	0.09	3
G-Strophanthin	NKAIN1					0.54	0.23	4

PIK3-MTOR pathway

name	target	ipNF05.5	ipNF06.2A	ipNF95.11b	ipNF95.6	ΔS mean	ΔS variance	num_lines
Tricirbine	AKT1					1.5	1.56	4
PF-05212384	MTOR					1.18	1.09	4
Torin-2	MTOR					0.72	0.51	4
PIK-294	PIK3CA	NA				1.03	0.57	3
CUDC-907	PIK3CA					0.93	0.77	4
PI-103	PIK3CA		NA			0.66	1.43	3
BYL-719	PIK3CA					0.55	0.16	4
PKI-402	PIK3CA					0.53	1.29	4
BAY-80-6946	PIK3CA					0.52	0.32	4

non-receptor protein kinase

name	target	ipNF05.5	ipNF06.2A	ipNF95.11b	ipNF95.6	ΔS mean	ΔS variance	num_lines
PF-3758309	PAK4					0.54	0.07	4
NCGC00344999-01	ITK					0.54	0.03	4
Englerin A	PRKCA					0.53	0.35	4
NVP-TAE226	PTK2					0.93	0.1	4
PD-166285	SRC				NA	0.52	0.47	3
PD-173955 Analogue 1	SRC				NA	0.5	0.44	3

other targets

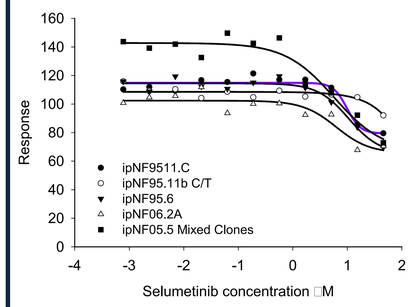
name	target	ipNF05.5	ipNF06.2A	ipNF95.11b	ipNF95.6	ΔS mean	ΔS variance	num_lines
Mithramycin	ADH1A		NA			0.76	0.06	3
Disulfiram	ALDH2		NA			1.36	2.53	3
Thapsigargin	ATP2A1					0.54	0.95	4
Methotrexate	DHFR		NA			0.58	0.1	3
GW-6471	PPARA	NA				0.64	0.01	3
Ivacomib citrate	PSMD1					0.71	0.24	4
AHPN	RARG					0.53	0.18	4
Gemcitabine	RRM2					1.09	0.63	4
Salinomycin	WNT1	NA				1	0.3	3
Embelin	XIAP		NA			1.71	0.05	3

no clear target

name	target	ipNF05.5	ipNF06.2A	ipNF95.11b	ipNF95.6	ΔS mean	ΔS variance	num_lines
Clofarabine						1.47	1.31	4
BTM-2C-dimer ketone				NA		1.17	0.31	3
Racecadotril			NA			1.05	0.34	3
Nanchangmycin						0.99	0.33	4
ciclopirox			NA			0.72	0.19	3
Monensin sodium salt						0.65	0.25	4
Flubendazole			NA			0.5	0.22	3
Fenbendazole						0.5	0.04	4

Top candidate compounds displaying drug-resistance in NF1 tumor cell lines. Blue data bars indicate the ΔS for each respective cell line (left to right) in each row.

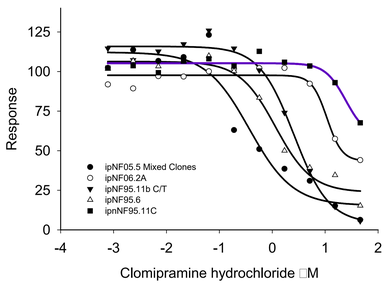
Selumetinib, the only approve NF1 drug has only moderate drug-sensitivity



	ΔS mean	ΔS variance	cell lines
Hypothemycin	-0.10	0.08	3
Selumetinib	-0.32	0.07	3
Cobimetinib	-0.09	0.01	4
BIX 02188	-0.33	0.04	4

Selumetinib had a modest drug-sensitivity, the purple line is the reference. The table shows additional modest sensitivity in similar drugs targeting MAPK1 (MEK1 Inhibitors).

Alternative drugs, like SSRIs have pronounced drug-sensitivity

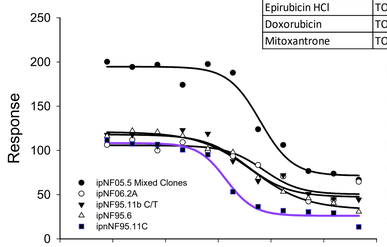


Clomipramine, a serotonin transport inhibitor drug displays sensitivity in tumor cell lines compared to reference. The purple line in the graph indicates reference cell line. The table shows ΔS_{mean} values for other similar SSRIs.

Results Cont.:

NF1 tumor cells are drug-resistant to anthracyclines, a common chemotherapeutic

name of compound	target	ΔS ipNF05.5	ipNF06.2A	ipNF95.11	ipNF95.6	log mean	variance	cell lines
Idarubicin HCl	TOP2A		NA			0.9	0.03	3
Epirubicin HCl	TOP2A					0.9	0.09	4
Doxorubicin	TOP2A					0.6	0.05	4
Mitoxantrone	TOP2A					0.6	0.08	4



NF1 tumor lines display resistance to anthracycline-type compounds. Anthracyclines are commonly used for MPNSTs, and other cancers. The purple line in the graph indicates the reference cell line. In the table the blue data bars reflect drug-resistance with positive ΔS values.

name	target	ΔS mean	variance	cell lines
Tricirbine phosphate	AKT1	**-1.51**	0.81	**4**
Clomipramine HCl	SLC6A4	**-1.35**	0.50	**4**
Geldanamycin	HSP90AB1	**-1.20**	0.79	**4**
Alvespimycin HCl	HSP90AB1	**-1.17**	0.65	**4**
SCH-900776	CHEK1	**-1.01**	0.16	**4**
Varlitinib tosylate	EGFR	**-0.92**	0.35	**4**
Piboserod HCl	HTR4	**-0.87**	0.51	**4**
A-443654	AKT1	**-0.82**	0.04	**4**
Fluvastatin	HMGCR	**-0.80**	0.04	**4**
Mycophenolic acid	IMPDH1	**-0.77**	0.04	**4**
IKK-2 Inhibitor VIII	IKBKB	**-0.71**	0.12	**4**
BMS-345541	IKBKB	**-0.71**	0.38	**4**
GNE-490	PIK3CA	**-0.66**	0.25	**4**
DCC-2036	ABL1	**-0.64**	0.12	**4**
Retaspimycin	HSP90AB1	**-0.57**	0.18	**4**
AIM-100	TNK2	**-0.54**	0.16	**4**
Temocapril HCl	ACE	**-0.53**	0.11	**4**

High confidence compounds displaying drug-resistance in four NF1 tumor cell lines.

name	target	ΔS mean	variance	cell lines
Secoisolariciresinol	AKT/NFKB1	**-2.15**	0.09	**3**
Duloxetine HCl	SLC6A4	**-1.17**	0.21	**3**
Shikonin	CCR5	**-0.97**	0.03	**3**
Verteporfin	YAP	**-1.03**	0.12	**3**
3-Methyladenine	PIK3CA	**-1.01**	0.16	**3**
Tivozanib	FLT1	**-1.22**	0.38	**3**
AZ 10606120	P2RX7	**-1.10**	0.31	**3**
CT-99021	GSK3B	**-1.07**	0.34	**3**
Chlorhexidine	MMP1	**-0.74**	0.11	**3**
Seliciclib	CDK1	**-0.59**	0.01	**3**
Amiodarone	---	**-0.63**	0.05	**3**
Mycophenolate mofe	IMPDH1	**-0.62**	0.06	**3**
Pitavastatin calcium	HMGCR	**-0.52**	0.01	**3**
Teriflunomide	DHODH	**-0.67**	0.21	**3**
Sepantronium bromid	BIRC5	**-1.07**	0.62	**3**
BMS-265246	CDK1	**-0.57**	0.14	**3**
Erlotinib HCl	EGFR	**-0.61**	0.26	**3**
Avobenzone	---	**-0.56**	0.20	**3**
AT-7867	AKT1	**-0.51**	0.16	**3**

Moderate confidence compounds displaying drug-resistance in three NF1 tumor cell lines.

Research Implications/Conclusion:

Tumor cell lines showed only moderate drug-sensitivity to the only approved NF1 drug Selumitinib. Furthermore, the NF1 tumor cell lines had drug-resistance to anthracyclines (i.e. doxorubicin) – a common class of chemotherapeutic for MPNST, and a variety of other metastatic cancers.

Noteworthy compounds with drug-sensitivity in NF1 tumor cell lines

- ***Statins – Fluvastatin, Pitavasta***
  - Simvastatin and lovastatin have been used in early clinical trials in NF1 children (autism, cognitive defects, attention impairment), 12 weeks (appeared effective) – longer study needed for tumor effects
- ***Mycophenolic acid and the prodrug mycophenlate mofetil***
  - Acts as Inosine 5'-Monophosphate Dehydrogenase (IMPDH) inhibitor
  - Use supported in a glioma meta-analysis - *DOI: 10.1007/s40265-021-01668-x*
- ***Secoisolariciresinol (SECO)*** (derived from flax seeds)
  - In clinical trial for breast cancer (Phase IIB)
  - In vitro SECO can re-sensitize cancer cells during doxorubicin chemotherapy *DOI: 10.7717/peerj.9163*
  - Decreases local inflammation, suppresses NFκB signaling, and inhibits mammary tumor growth in mice *DOI: 10.1007/s10549-018-5021-6*
- ***Verteporfin***
  - In vitro can effectively sensitize NF1 related pNF tumor cells to selumetinib *doi: 10.7150/ijms.78386*
  - inhibits growth of human glioma in vitro without light activation *doi.org/10.1038/s41598-017-07632-8*
  - Photoactivated porphyrin In clinical trials - NCT04590664, Verteporfin for the Treatment of Recurrent High Grade EGFR-Mutated Glioblastoma
- ***SSRIs - Clomipramine , Duloxetine***
  - Serotonin receptor signaling in cancer cells is a known mTOR mechanism *doi: 10.7150/thno.55986*

Future Work/Acknowledgements:

We are currently expanding the work to include drug combinations. We are looking at a Bayesian approach for the biomarker Chromosome 8q-gain associated with MPNSTs – and have a drug list for this biomarker.

We would like to thank the Children's Tumor Foundation for award funding for this project as part of our incubation prize for winning the 2022 Hack4NF competition.